2

Introduction to the science

INTRODUCTION

2.1 Chapters 2, 3 and 4 cover the scientific processes and related issues underlying cloning and stem cell technologies. This chapter gives a background to the field of reproductive and developmental biology and the technologies relevant to the cloning of human tissues, organs or whole individuals. It provides a basis for the scientific developments, ethical issues and regulatory options discussed in the rest of the report.

2.2 A glossary of scientific terms is provided at the end of this report [Appendix E]. The glossary is based on that used by AHEC.[1] Where new terminology has arisen from research over the past three years, the glossary developed by the Australian Academy of Science[2] is used. A few definitions of basic terms are from Collins Dictionary of Biology.[3] Many definitions are changing as new research revises understanding of reproduction and development. The key issues and terminology are presented in the diagrams at the end of this chapter.

2.3 Research in molecular, cellular and developmental biology is progressing at extraordinary speed and challenging previous understanding of cell, tissue and embryo development. The future directions of this research, the potential for revolutionising aspects of medicine and health care, and the role of Australian scientists in these developments is the subject of Chapters 3 and 4.

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1 AHEC report, Appendix 3, p.50

2 *Human Stem Cell Research*, Australian Academy of Science, 18 April 2001

3 Hale, WG, and Margham, JP, *Dictionary of Biology*, Collins, Glasgow, 1988

REPRODUCTIVE BIOLOGY—BACKGROUND

2.4 Knowledge of human reproduction and the molecular, cellular, hormonal and other factors that regulate the development of sperm and egg (gametes), fertilisation, pregnancy, embryo and foetal development, has accumulated over the past seventy years. Current developments in research, including those covered in this report, depend fundamentally on this background of knowledge. Traditionally, such knowledge has been gained first by studying reproductive processes in apparently simple systems such as those of amphibians (including frogs and toads), fish and a range of mammals including the rodents (mice, rats), agricultural livestock (sheep, cattle, pigs) and primates (marmoset, rhesus monkey, baboon) with validation in the human as appropriate. The developmental mechanisms that regulate reproduction in primates and humans are similar, whereas in other species some mechanisms may have significant differences.

2.5 The development of this and any scientific field depends on experiments that are carried out with laborious precision. The process includes a careful review of the background knowledge, definition of a question, formulation of an hypothesis and design of an experiment to test this hypothesis. The results are published after critical review by peers in the field.

2.6 Much of scientific debate concerns the validity of experiments and the interpretation of results. This rigorous approach is essential if robust results are to be obtained. The scientific approach has tended to be tested first on animal model systems before being extended to the human, to reduce the risk of unpredictable side effects. Results from such studies over the past fifty years have provided new treatments for infertility, the regulation of fertility, assisted reproductive technologies and now cloning technologies.

2.7 The field of human reproduction and assisted reproductive technologies is an area where scientific method needs special care for a variety of moral, legal and social reasons. However, at some point a leap has to be made to the human from the earlier, animal work. This leap was made in the development of *in vitro* fertilisation (IVF), resulting in the birth of Louise Brown[4] in 1978, and of about 350,000 IVF babies born since then.[5] Because of the worldwide focus of research in this area in the past twenty years, in which Australian scientists have played a prominent role, there is now

4 Joint Report of Human Fertility and Embryology Authority and Human Genetics Advisory Commission, UK, in *Cloning Issues in Reproductive Science and Medicine*, 1978, paragraph 3.1

5 Some press estimates are as high as 1 million.

more information about human gametes and embryos than about other primates.

2.8 Consequently there is a strategic point when deciding if research is best pursued in a rodent or primate model system, or if it is better pursued directly in the human. This is now the case with some assisted reproductive technologies where carrying out research in animal models would be a reversion to a less understood system. In practice, research is normally carried out in parallel in humans and animals.

Outline of Human Reproductive Processes

2.9 The female human reproductive tract includes the ovary, oviduct (fallopian tube) and uterus (Figure 1).[6] Eggs grow in the ovary, regulated by circulating hormones. Usually, one egg is released each menstrual cycle in the human female (ovulation). This egg passes into the oviduct. Fertilisation can occur approximately twenty four hours later if sperm are present. The sperm and egg fuse in the process of fertilisation.

2.10 The male human reproductive tract includes the testis, epididymis and vas deferens, with the prostate, seminal vesicles and Cowper's glands. Sperm are produced in the testis, matured and stored in the epididymis and tens of millions are released at ejaculation.

2.11 The process of fertilisation includes the attachment of sperm to the membrane surrounding the egg (zona pellucida); the penetration of the egg by the sperm; the migration of the sperm head across the cell and fusion of the sperm and egg nuclei. Fusion occurs when the chromosomes from the sperm and egg align to form the new embryo (syngamy). During this process, from the penetration of the zona pellucida by sperm to syngamy, the cell is called a zygote.[7] Once syngamy is completed the cell is referred to as an embryo.

2.12 The embryo divides (2, 4, 8, 16 cells etc) as it passes through the oviduct, arriving in the uterus about day 4 as a 'morula', a ball of 32-64 cells (Figure 2). Once in the uterus the morula develops into a 'blastocyst' (by day 5-6) which consists of an outer casing of cells that will become the placenta, and an inner cell mass that will become the foetus.

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6 The terms used in this section are explained in the glossary at the end of this report

7 The Infertility Treatment Act (Vic), section 3 defines an 'embryo' as 'any stage of human embryonic development at and from syngamy'. Syngamy is defined as 'that stage of development of a fertilised oocyte where the chromosomes derived from the male and female pronuclei align on the mitotic spindle'. The term zygote means 'the stages of human development from the commencement of penetration of an oocyte by sperm up to but not including syngamy'

2.13 Consequently, the first week of pregnancy is a dynamic period when the embryo develops from a single cell, resulting from the fusion of sperm and egg, to a blastocyst of 100-200 cells (Figure 3). At about day 6-7, the blastocyst 'hatches' from the zona pellucida and becomes attached to the inner lining of the uterus. At this point of early implantation the placenta starts to form and to invade the blood supply of the mother in order to gain nourishment. At about 14 days the primitive streak is formed (see below). Implantation of the embryo continues until the pregnancy becomes fully established (Figure 3).

2.14 Although precise data is difficult to obtain, it is estimated that up to half of all naturally formed embryos fail before the full establishment of pregnancy.[8] The reasons for these failures are obscure and almost impossible to study in the human, but are thought to be due to genetic abnormalities in the embryo (about 30%), inadequate synchrony or development of hormonal signals between the embryo and the mother (about 30%), with the remainder due to unexplained causes.

2.15 It is at this early stage, during the first two weeks of pregnancy, that twins can be formed. Non-identical twins are the result of two eggs being fertilised and both of these implanting. Identical twins (natural human clones) are the result of a splitting of the single embryo at some stage during the first 2 weeks of its development.

2.16 Fourteen days is the approximate time when the primitive streak, the first confirmation of the organised embryo and its orientation, is formed. This is when 'identical' (monozygotic) twin embryos derived from a single egg first become evident and also when implantation and pregnancy are becoming more established. This was one reason that 14 days was adopted by the Warnock Committee and by the legislature in the United Kingdom as the limit for the period when research on embryos is permitted.[9] In Australia, 14 days is also the limit for research on embryos in Western Australia, South Australia and in the guidelines of the National Health and Medical Research Council (NHMRC).

Assisted Reproductive Technologies

2.17 During the last thirty years a significant field of medicine has developed, aiming to help infertile couples achieve successful birth. In general, the techniques have resulted from the close study of the normal process,

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8 Wood, J.W., 1989, Fecundity and actual fertility in humans, *Oxford Reviews of Reproductive Biology*. 11, pp.61-109; Wilcox, A. J. *et al*, 1988; Incidence of early loss of pregnancy. *New Engl. J. Med.* 319, pp.189-194

9 *Report of the Committee of Inquiry into human Fertilisation and Embryology* (1984), known as the Warnock Report, www.doh.gov.uk/bus guide/hfea/page1.htm

outlined above, and then a series of interventions to enhance or replace the factors that are inhibiting fertility in the male or the female.

2.18 The causes of infertility in men and women can be obscure, but many factors are known. These can be physical, such as blockage or damage in the male or female tract, genetic, hormonal, biochemical or metabolic, resulting in inadequate growth and maturation of the sperm or egg or failure of embryo development and the establishment of pregnancy.

2.19 About 350,000 successful births around the world have been achieved by a wide range of assisted reproductive techniques. The overall success rate of these assisted procedures is still low, with babies born averaging fewer than 20% of attempts made, although some clinics claim to achieve over 30% success. One should remember that the overall success of natural fertilisation and implantation is estimated as little more than 50%.[10]

2.20 As a result of all this work it is now routine to collect eggs from the ovary of women and sperm from men. The process of egg collection in humans requires a course of hormone treatment during the first two weeks of the menstrual cycle to stimulate multiple egg development. Egg development is monitored by ultrasound scanning and blood samples are taken for hormone measurement. Eggs are collected in one of two ways: using a needle guided by ultrasound (under a general anaesthetic or a mild sedative), or by laparoscopic surgery. During this latter procedure which normally requires a general anaesthetic, a small incision is made in the abdomen through which the laparoscope is inserted. This enables the surgeon to see into the abdomen and locate the mature eggs in the ovary. A fine, hollow needle is inserted at another site and the eggs are collected by suction. There may be minor side effects, discomfort or complications during this process.[11]

2.21 Sperm and eggs can be maintained in appropriate culture conditions, frozen and stored, used to carry out fertilisation *in vitro* (outside the body); or for transfer of sperm, eggs, or embryos back into the female reproductive tract for pregnancy to be established.

2.22 One result of the success of these procedures is that more embryos may be produced than are required for replacement into the woman's uterus. These embryos are frequently stored frozen for possible later use by the parents. However, many thousands of these surplus embryos are not

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10 Wood, J. W., 1989, Fecundity and actual fertility in humans, *Oxford Reviews of Reproductive Biology*. 11, pp.61-109; Wilcox, A. J. *et al*, 1988, Incidence of early loss of pregnancy, *New Engl. J. Med.* 319, pp.189-194

11 The egg donation procedure is outlined in a leaflet—'Egg Donation'—produced by the Human Fertilisation and Embryology Authority in the United Kingdom http://www.hfea.gov.uk/egg/eggdon.htm

required.[12] A few of these embryos have been used to isolate embryonic stem cells from the inner cell mass, giving rise to the current explosion of interest in the field. The nature and use of embryonic stem cells is discussed further below.

2.23 Another assisted reproductive technique, pre-implantation genetic diagnosis or cell biopsy, involves the removal of a single cell from a multi-celled embryo after *in vitro* fertilisation. This cell may be analysed to diagnose some genetic abnormalities or physiological deficiencies, without damaging the whole embryo. The technique can be applied when there is a family history of a genetic disorder that is expressed in a proportion of the embryos and allows the identification of normal embryos for transfer to the mother. Similar techniques could be used to screen stem cells for some defects.

The Structure and Life Cycle of a Cell

2.24 Cells are the building blocks of the body, usually specialised for their role in particular tissues and organs (for example, nerve cell, muscle cell etc). Cells have limited life spans and are replaced throughout life by new cells, generated within the tissue or organ. Cells are made up of the nucleus, which contains the chromosomes carrying genetic information, and the cytoplasm. The cytoplasm contains mitochondria which contain a few genes and are responsible principally for energy and cell regulation. (Figure 4)

2.25 All cells form initially from unspecialised cells. In the embryo, stem cells form the early tissues and organs. Under the influence of unknown genetic and chemical signals, cells become specialised and differentiated. Some stem cells are retained in most tissues or organs throughout life to participate in regeneration and repair.

2.26 Until relatively recently, once cells embarked on the various pathways towards specialisation, such as muscle, nerve, liver etc, they were thought to be 'committed' and irreversibly locked into that particular cell type. Although each somatic cell contains a full set of chromosomes and genes (genome) of the individual, only the genes that are required for that cell's particular function are expressed (selectively activated).

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12 Tara Hurst and Paul Lancaster, *Assisted Conception Australia and New Zealand 1998 and 1999*, Australian Institute of Health and Welfare National Perinatal Statistics Unit and the Fertility Society of Australia, AIHW National Perinatal Statistics Unit, Sydney, 2001, p.7. The number of embryos that are frozen each year exceeds the number thawed so the total number of embryos in storage continues to increase. The number of embryos in storage has nearly trebled since 1994 from 22,280 in 1994 to 65,518 in 1999

2.27 Recent research shows that, under certain conditions, cells are much more flexible than was thought. For example, a muscle cell can be 'deprogrammed' and turned into a nerve cell when transplanted into nerve tissue. This process is known as 'transdifferentiation' and scientists are only now starting to understand how it is achieved since it implies the reprogramming of the cell and activation of other parts of the genome to form a different cell type. Embryonic stem cells or adult stem cells can develop more easily down different pathways, since they have yet to become fully specialised and committed to a single pathway.

2.28 Most of the cellular triggers and signals that determine the choice of pathway, that is, towards muscle, nerve etc are not known and this is where a lot of the international effort in cloning technologies and stem cell research is now focused.

2.29 This flexibility introduces the concept of cell 'potency' or potential (Figure 5). There are 'totipotent' cells, which can develop into a whole individual, such as a fertilised egg or the individual cells of the embryo up to the 16-32 cell stage. There are also 'pluripotent' cells, such as embryonic stem cells (ES cells) and embryonic germ cells (EG cells) which can develop into many or all of the cells or tissues of the body but not into a whole individual. There are also 'multipotent' cells, such as adult stem cells that can develop into a more restricted range of tissues or organs. The terms pluripotent and multipotent are often used as synonyms.

Somatic cells and germ cells

2.30 The human has two fundamentally different cell types:

- germ cells, located in the gonads (ovary and testis). These are the cells from which sperm and eggs arise; and
- somatic cells, which are all other cell types of the body.

The genome in germ cells is transmitted, after fertilisation of the egg by the sperm, to future generations. Therefore genetic manipulation of germ cells results in the modified genome being transmitted to future generations. In contrast, the genome in somatic cells is not transmitted to future generations and genetic manipulation of a somatic cell only affects the genome of that cell.

CLONING

The Definition Of Cloning

2.31 There are many definitions of cloning in plants and animals. Cloning occurs naturally in the asexual reproduction of plants, the budding of yeast in beer, the formation of identical twins and the multiplication of cells to repair damaged tissue in the normal process of healing. Cloning techniques in plants have been in widespread use for centuries in gardening and crop development. Lower vertebrates such as earthworms or flat worms, when cut in half, will regenerate two genetically identical individuals.

2.32 Cloning can also be achieved through artificial technologies. It is possible to clone DNA, cells, tissues, organs and whole individuals. The method of nuclear transplantation, (now known as somatic cell nuclear transfer) which was developed first about 40 years ago in frogs, has now been adapted successfully to make clones of mice, sheep, goats, pigs and cattle. Rhesus monkeys have been cloned by embryo splitting techniques.

2.33 **Therefore, it is important to note that cloning does not necessarily mean the replication of an entire individual. This, however, is often the public perception, reflected in the Australian and international media.**

2.34 The AHEC report defines cloning as 'asexual propagation without altering the nuclear genome'.[13] There is little distinction in the AHEC report between the different purposes for cloning, for example, cloning for reproductive purposes or cloning for the purposes of therapy. The isolation of human embryonic stem cells, which opened the possibility of such cell therapy, was achieved just before the AHEC report was published in December 1998.

2.35 However, the AHEC report does distinguish between procedures for the cloning of a whole human individual and the copying of the component parts of a human (such as DNA and cells).[14]

2.36 An alternative definition of 'cloning' to that in the AHEC report was developed by the Australian Academy of Science. The Academy published a position statement *On Human Cloning* in February 1999[15] and held an international symposium *Therapeutic Cloning for Tissue Repair* in

13 AHEC report, Glossary

14 AHEC report, E3 p.iv

15 *On Human Cloning*. A Position Statement, 4 February 1999, Australian Academy of Science

September 1999.[16] The Academy published a statement *Human Stem Cell Research*[17] in April 2001. The following working definitions were used:[18]

- cloning: the production of a cell or organism with the same nuclear genome as another cell or organism;
- reproductive cloning: to produce a human fetus by nuclear replacement; and
- therapeutic cloning: to produce human stem cells, tissues and organs.

These definitions recognise the different purposes for cloning and distinguish between the cloning of a whole human individual and cloning of cells and tissues.

2.37 Therefore, cloning can mean different things to different people. Moreover, there is overlap between the definitions for reproductive cloning and for therapeutic cloning, since in both an embryo may be formed or used for research. The Committee acknowledges that existing definitions are confusing.[19]

Cloning Technologies

2.38 Two major scientific breakthroughs have shaped the recent developments in cloning technologies. The first, somatic cell nuclear transfer, is represented by the 'Dolly' experiment reported in 1997.[20] The second, the isolation and characterisation of human embryonic stem cells was reported in 1998.[21]

Somatic cell nuclear transfer

2.39 For many years attempts to clone mammals by nuclear transplantation were unsuccessful, possibly because the nuclei were usually placed in fertilised rather than unfertilised eggs.[22] In 1996 Dr Ian Wilmut and his colleagues cloned the first mammal produced from a sheep foetal skin cell fused with an oocyte.[23] Their subsequent experiments resulting in Dolly produced the first mammal cloned from a fully differentiated adult somatic cell.

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16 *Therapeutic Cloning for Tissue Repair*, Report from a Forum held on 16 September 1999, Australian Academy of Science

17 *Human Stem Cell Research*, 18 April, 2001, Australian Academy of Science

18 *On Human Cloning*, A Position Statement, 4 February 1999, Australian Academy of Science, pp.7-8

19 See Chapters 6 and 7 for further discussion of reproductive and therapeutic cloning

20 Wilmut, I. *et al*, *Nature*, Volume 385, 1997, pp.810-13

21 Thomson, James A. *et al*, *Science*, Volume 282, 6 November 1998, pp.1145-1147

22 Gurdon J.B. and Colman A., *Nature*: Volume 402, 16 December 1999, p.744

23 Campbell *et al*, *Nature*, Volume 380, 7 March 1996, pp.64-66

2.40 In the Dolly experiment, a cell from the mammary gland of an adult sheep was fused, by means of an electric pulse, with an unfertilised, enucleated (nucleus removed) egg from a second sheep. The resulting fused cells developed into an embryo which, after transfer into the uterus of a third sheep, developed into a whole individual (Figure 6). This new sheep known as Dolly, born after a normal pregnancy, has lived an apparently normal life and produced lambs. Dolly was the only lamb born from 277 attempts. The cloning procedure, where the nucleus of a somatic cell is transferred into an unfertilised enucleated egg, is now known as 'somatic cell nuclear transfer'.[24]

2.41 This experiment proved that an adult cell could, under certain circumstances become totipotent, form an embryo and develop into a whole individual—a result that could not have been predicted from earlier understanding of mammalian embryonic cell specialisation and commitment.

2.42 The Dolly experiment raised the possibility that human reproductive cloning is feasible. However, many of the 277 attempts to clone Dolly resulted in abnormal placentas and foetuses and other complications during pregnancy or at birth. Similar failure rates and abnormalities have resulted from attempts at cloning other animals.

Embryonic stem cells

2.43 The second major scientific breakthrough to shape recent developments in cloning technologies has been in the isolation and characterisation of human embryonic stem (ES) cells. These pluripotent cells can be grown to produce cell lines and tissues with the aim of treating disease or perhaps growing organs for transplantation, an application referred to as 'therapeutic' cloning.

2.44 During the early development of an embryo, the morula develops into a blastocyst (Figure 3). The cells of the blastocyst are specialised into an outer casing of cells that will become the placenta, and an 'inner cell mass' that will eventually become the foetus. The outer cells are now 'committed' to become placental tissue and have lost the ability to develop into other tissues and organs. However, the inner cell mass is composed of embryonic stem cells, which retain the ability to become many specialised cells or tissues.

2.45 Embryonic stem cells can be removed from the blastocyst with a thin glass needle (pipette), or by a biochemical dissociation of the cells (Figure 7). They can then be placed into culture medium, and can replicate, retaining

24 Wilmut, I. *et al*, *Nature*, Volume 385, 27 February 1997, p.810

their pluripotent capacity indefinitely. They may be frozen and stored. Stem cells can be grown in culture, differentiating into a wide range of specialised cell lines.

2.46 The first embryonic stem cells from mammals were isolated from mice in 1981.[25] It took 14 years more before embryonic stem cells were isolated from non-human primates, this delay being due largely to the difficulties associated with obtaining monkey embryos in sufficient numbers for experimental research. The breakthrough came with the rhesus monkey in 1995,[26] followed by the marmoset monkey in 1996.[27] The same techniques were then applied in the USA to 14 human blastocysts produced by IVF, donated with informed consent, resulting in the isolation of five human embryonic stem cell lines in 1998.[28] Concurrently, the isolation of pluripotent human embryonic germ cells was reported in 1998.[29]

2.47 Research in human embryonic stem cell biology has spread rapidly in Australia, Europe and the USA, with significant research teams also in Israel and Singapore. There was an immediate global debate initiated about the science and ethics of reproductive and therapeutic cloning. The ethical and regulatory dimensions of this debate are addressed in Chapters 5-12 of this report.

Adult stem cells

2.48 Just as the embryo contains stem cells that may take different paths to build tissues and organs, stem cells remain present in the body throughout life and are responsible for normal repair and replacement in the different tissues and organs. These 'adult' stem cells are thought to have less flexibility. For example, blood stem cells in the bone marrow have the ability to develop into all of the various blood cells. The identification of such stem cells in muscle, brain, liver, pancreas and other tissues is in its early stages but research is progressing rapidly.

2.49 To date, it has proved difficult to routinely identify adult stem cells from the majority of organs. It has not proved easy to grow such cells or to maintain them in an undifferentiated state in culture, because they naturally incline to become one or other more specialised cell type such as muscle, nerve or skin.

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25 Martin, G., 1981, *Proc. Nat.. Acad. Sci. USA* 78, pp.7634-7638

26 Thomson, J. A. *et al.*, 1995, *Proc. Nat. Acad. Sci. USA.* 92, pp. 7844-7848

27 Thomson, J. A. *et al.*, 1996, *Biology of Reproduction* 55, pp.254–259

28 Thomson, J. A. *et al. Science*, Volume 282, 6 November 1998, pp.1145-1147

29 Shamblott, M. J. *et al.*, 1998, Derivation of pluripotent stem cells from cultured human primordial germ cells, *Proc. Nat. Acad. Sci. USA* 95, pp.13726-13731

2.50 Figure 8 shows an idealised adult stem cell therapy. An adult cell from a patient is reprogrammed, and perhaps genetically manipulated, before being cultured to the required cell type and transplanted back to the patient. At present these procedures may require a somatic cell nuclear transfer stage, passing through an embryo phase. In future it may be possible to reprogram the cell without an embryo phase.

2.51 In the past two years there has been a major expansion in research on adult stem cells. There is a new understanding of their flexibility and potential but current knowledge suggests it is unlikely that these will be as broad as in embryonic stem cells. A summary of the procedures employed for somatic cell nuclear transfer, embryonic stem cell and adult stem cell research is provided in Figure 9.

Subsequent developments

2.52 The cloning procedures that resulted in Dolly have been extended to other mammals but not to humans. There is a low success rate and many embryos transferred to surrogate mothers die during pregnancy, others at birth, many with serious abnormalities. In general terms, the success of the somatic cell nuclear transfer procedure to form a viable blastocyst is approximately 1-2% of attempts made. The success of cloned embryos transferred to the uterus resulting in live births is also of this order. The reasons for the many failures have yet to be fully defined. The efficiency of the procedure must be improved greatly before it becomes a viable technique, either for animal husbandry or for cell manipulation.

2.53 An example of the application of somatic cell nuclear transfer technique is provided here. Whilst it illustrates the developments it was not an area on which the Committee received evidence. In 1997, Dr Wilmut's group cloned a sheep, Polly, from a foetal skin cell into which a human gene for a valuable pharmaceutical protein, the human clotting factor IX had been inserted. Factor IX could subsequently be extracted from Polly's milk and concentrated for potential use in treating human haemophiliacs.[30] This new source of factor IX avoids the risk of human blood products transmitting viruses such as AIDS or hepatitis C.

2.54 Applications of somatic cell nuclear transfer and other cloning technologies in the near future may include, for example, the production of animals that generate valuable pharmaceuticals in their milk or urine, or produce milk or meat with enhanced nutritional value.

30 McLaren, A., *Science*, Volume 288, 9 June 2000, 1779

Potential benefits of stem cell therapy

2.55 A great deal of research is focusing on how undifferentiated embryonic or adult stem cells can be induced to develop into one or other tissue and organ cell lineage. This 'cell lineage choice' will determine whether they become brain, muscle, gut, liver, pancreatic cells or so on. The ability to control and direct cell differentiation or to identify the factors responsible for doing so, has enormous potential for new therapies in medicine and for new biomedical industries. The prospects include banks of cells that are tailored for specific diseases in specific people. The intellectual property associated with the factors that determine cell choices will be valuable. Consequently, there is intense competition in laboratories around the world to elucidate the process and to patent this new knowledge.

2.56 The scientific competition to understand the factors regulating cell differentiation emphasises the urgency of this research. The potential benefits include a complete revolution in the ability to treat acute and chronic diseases, including Alzheimer's, Parkinson's, diabetes and many others. These applications may derive from the use of embryonic stem cells, adult stem cells or the factors that regulate their differentiation.

2.57 A hypothetical example of embryonic stem cell therapy is the treatment of Type 1 diabetes. Using somatic cell nuclear transfer, the nucleus of a somatic cell from a patient with the disease could be fused with an enucleated donor egg. The cell would develop into a blastocyst from which inner cell mass cells could be isolated and grown in culture with growth factors, as yet unknown, to develop into pancreatic islet cells that produce insulin. Because these cells came from and are genetically identical to the patient (except for mitochondrial genes in the cytoplasm of the donor egg) they would not be rejected when transplanted back into the patient. There would be little or no need for immune-suppressing drugs, with their often unpleasant and serious side effects.

2.58 Adult stem cells could provide an ideal cell therapy if it were possible to identify and isolate them from a specific tissue or organ type, multiply and grow them in culture, manipulate them to repair any genetic or metabolic deficiency and store them until required. When using these cells to repair damaged organs, including the brain (Parkinson's or Alzheimer's), they could be transplanted back into the same person following manipulation. The cells would be fully matched and compatible since they would have been specifically designed or enhanced for the person and that disease.

2.59 However, a great deal of research is required before applications of stem cell biology to diabetes and other diseases will become available. Diabetes may also prove intractable to such treatments; and in this and other

diseases the replacement of cells may not necessarily cure the disease. Indeed, the new cells may be vulnerable to the original disease process, but they may provide a temporary solution.

2.60 While stem cell therapies are unlikely to be widely available for 5–10 years, the potential cost savings of therapies arising from stem cell research, including the use of newly discovered cell signals and triggers that regulate differentiation, is enormous. The health care implications are considerable.

Other Related Technologies

2.61 Applications arising from research in somatic cell nuclear transfer, embryonic stem cells and adult stem cell will become a major field of biomedical science. In addition, there are other methods and variants that may lead to new cloning technologies. These include embryo splitting, cross species cell transfer and mitochondrial transfer. These are not the focus of this report and are summarised only briefly below because of recent press references.

Embryo splitting

2.62 During the first few days after fertilisation, the morula, a ball of 32-64 cells, enters the uterus and develops into a blastocyst. All of the individual cells (blastomeres) up to the morula stage are thought to be totipotent. They can be separated singly or in multiple cell groups each of which can develop to form a whole new individual. This procedure has now been achieved routinely in rodents, sheep and cattle and was demonstrated recently in the rhesus monkey.

2.63 In addition, the morula and the blastocyst may be bisected, the latter requiring the presence of some inner cell mass cells, and each half may develop into a separate individual. This procedure has been performed but is not routine. Natural embryo splitting is known to occur in the formation of identical twins (natural clones) in humans.

Cross species cell transfer

2.64 The transfer of DNA, cells, tissues or organs between species is known as xenotransplantation. Examples of this research include somatic cell nuclear transfer of an adult cell from one species into an egg of another. Some of these attempts have resulted in the formation of embryo-like structures, used as a potential source of embryonic stem cells.

2.65 A further example is in organ transplantation, where an animal heart, liver, or kidney is used as a short-term transplant until a human organ

becomes available. This may require genetic manipulation to facilitate acceptance of the organ. A risk of these procedures is the inadvertent transfer of known and unknown viruses or other infectious agents. The Committee's recommendation on cross species research is in Chapter 12, at Recommendation 11.

Ooplasmic transfer

2.66 Recently, about 30 births were reported in the press[31] and in *Human Reproduction*[32] after the eggs of the mothers had been injected with additional cytoplasm from a donor egg. The objective was to overcome infertility in the mothers. The donated cytoplasm included mitochondria, small cellular organelles that contain a few genes that are responsible for energy regulation in the cell. There is no direct evidence that this treatment overcomes infertility. Many in the scientific community have questioned the approach, which had not been fully tested in animals. Subsequent reports have revealed that 2 of the 17 foetuses formed by the technique at one medical centre had Turner's syndrome, a serious chromosomal abnormality.[33]

SUMMARY

2.67 Any review of the scientific literature, or indeed the popular press, over the past two years will confirm the extraordinary pace of research in this area. The methods for isolating embryonic, adult and other cells are being refined. The chance of success, one in 277 in the case of Dolly, is being improved. However, the field is still young and many procedures are proving difficult to repeat. Undoubtedly, progress in the next five years will accelerate with further breakthroughs. These developments will need to be monitored as they will have repercussions on the related ethical and regulatory dimensions of cloning and stem cell technologies.

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31 BBC News, 4 May 2001

32 Mitochondria in human offspring derived from ooplasmic transplantation: Brief communication, J.A. Barritt *et al, Human Reproduction*, Volume 16, No.3, pp.513-516, March 2001

33 *Sydney Morning Herald*, 21 May 2001, p.5

Figure 1. The human female reproductive tract

Eggs grow in the ovary, stimulated by circulating hormones. Usually, one egg is released each cycle in the human female (ovulation). This egg passes into the oviduct where fertilisation may take place. The fertilised egg starts to divide as it passes through the oviduct, arriving in the uterus as a 'morula' of about 30-60 cells as shown in Figure 2. Once in the uterus the morula develops into a 'blastocyst' which consists of an outer casing of cells that will become the placenta, and an inner cell mass that will become the foetus.

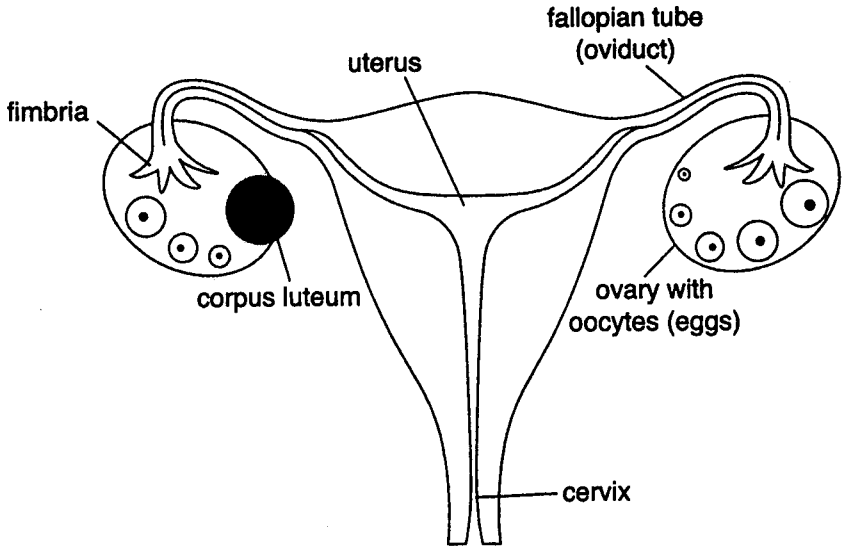


Figure 2. Development of the human embryo in the reproductive tract, from fertilisation to implantation

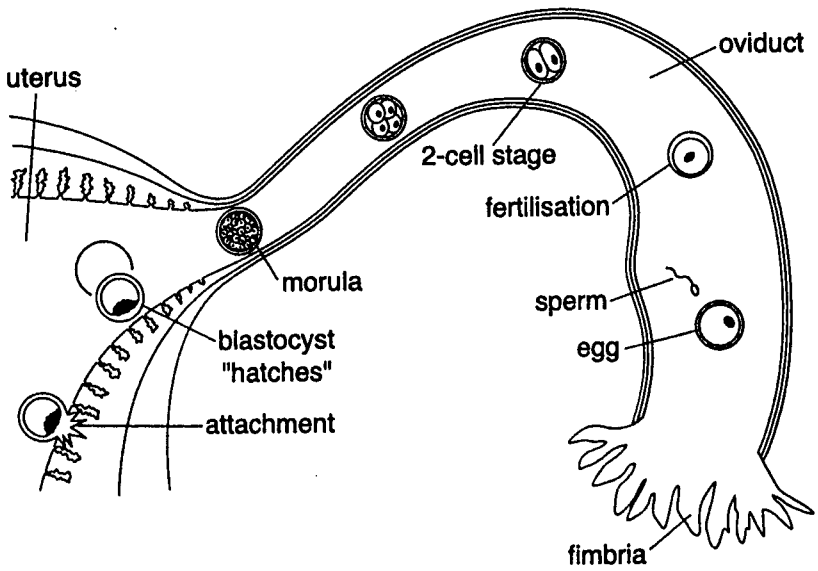


Figure 3. Early embryo development

In the uterus, the blastocyst 'hatches' from its surrounding membrane called the zona pellucida on about day 6-7. The hatched blastocyst then attaches to the inner lining of the uterus. Immediately, cells project out to invade the uterus and connect with the maternal blood supply so that nutrients will flow to sustain the embryo. This process of attachment and invasion is called implantation.

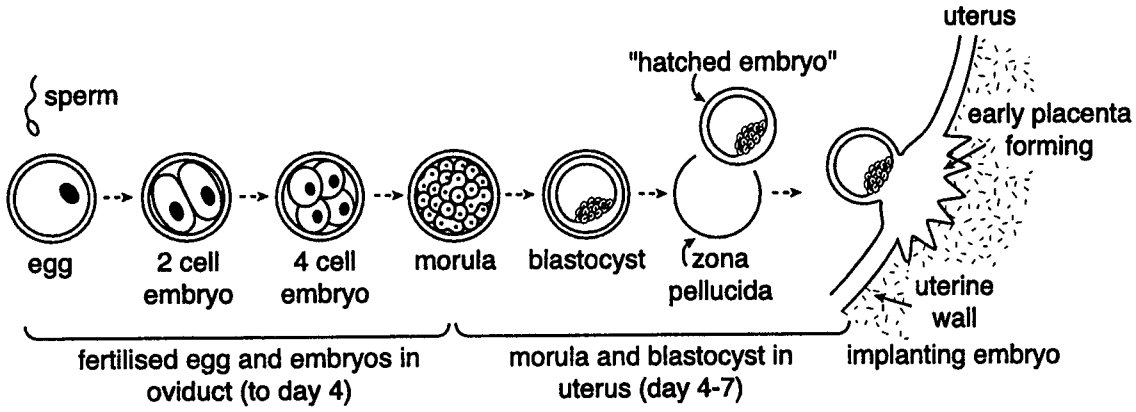
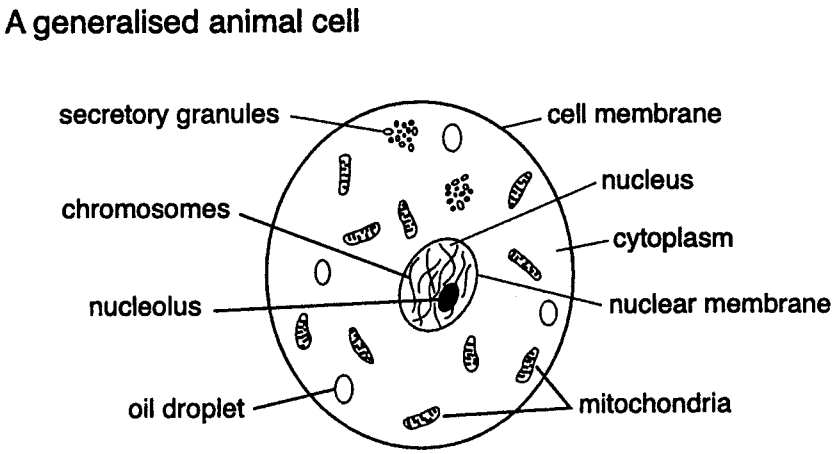


Figure 4. The Cell



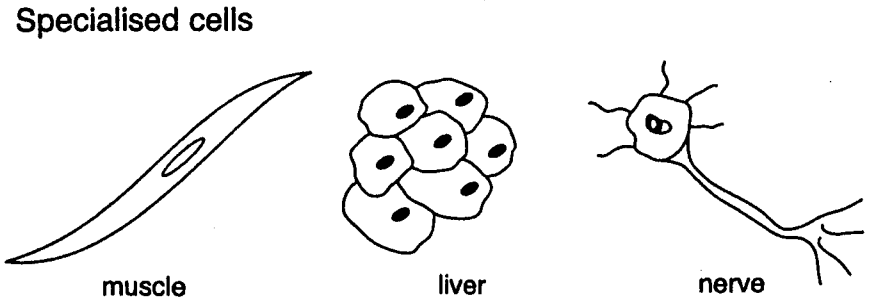
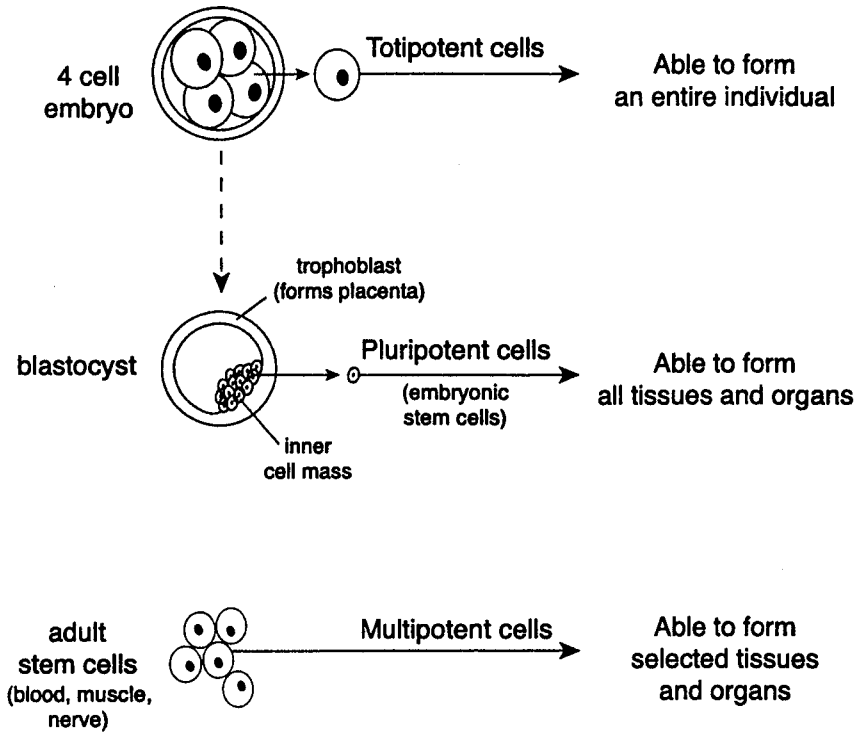


Figure 5. Cell Potency



Note: The terms "pluripotent" and "multipotent" are often used synonymously.

Figure 6. Somatic Cell Nuclear Transfer

An adult cell (eg. mammary cell, in the case of 'Dolly'). is fused, using an electric pulse, with an egg from which the nucleus has been removed. The resulting 'embryo' is then transferred to the uterus. In the case of Dolly, only one lamb was born from 277 attempts. The cell treated this way has been 'reprogrammed' by as yet unknown factors in the egg cytoplasm to becomes totipotent (capable of developing into a whole individual).

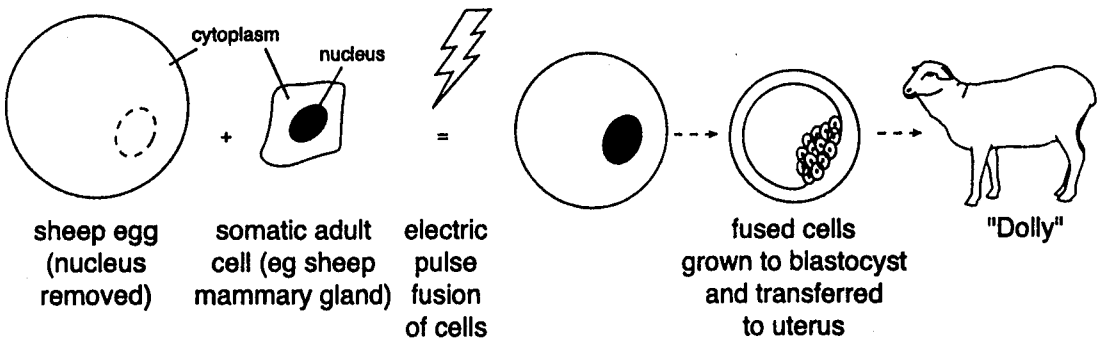


Figure 7. Embryonic Stem Cells

Embryonic stem cells (ES) cells are isolated from the inner cell mass of the blastocyst. They can be grown and multiplied indefinitely in culture without differentiating, but can be made to differentiate into a range of cell types. The factors determining 'cell lineage choice' ie. whether cells grow into brain, liver, muscle, gut etc. are not yet understood and are the focus of much current research. At present, embryonic stem cells can only be derived from the inner cell mass of the blastocyst and the procedure destroys the blastocyst. Embryonic stem cells are multipotent/ pluripotent (they can form many of the cells or tissues of the body) but not totipotent (they cannot form a whole individual).

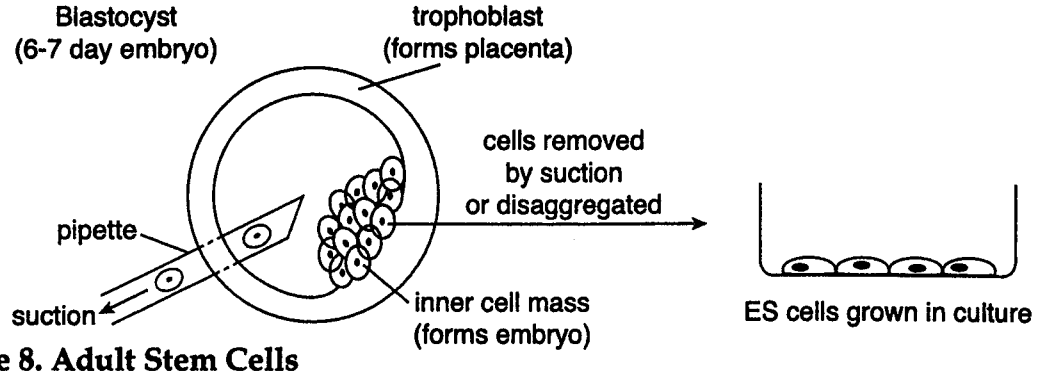


Figure 8. Adult Stem Cells

Stem cells are present in the foetus, child and adult. These 'adult' stem cells have a limited ability to differentiate into various cell types. They are responsible for normal cell replacement and wound healing. An example is the blood stem cell, present in the bone marrow, which replaces blood cells throughout life. Currently there is no routine way to identify adult stem cells in tissues and organs and this is the focus of much research. Successful identification and multiplication of adult stem cells would allow the development of stem cell therapies that do not require the use of embryos.

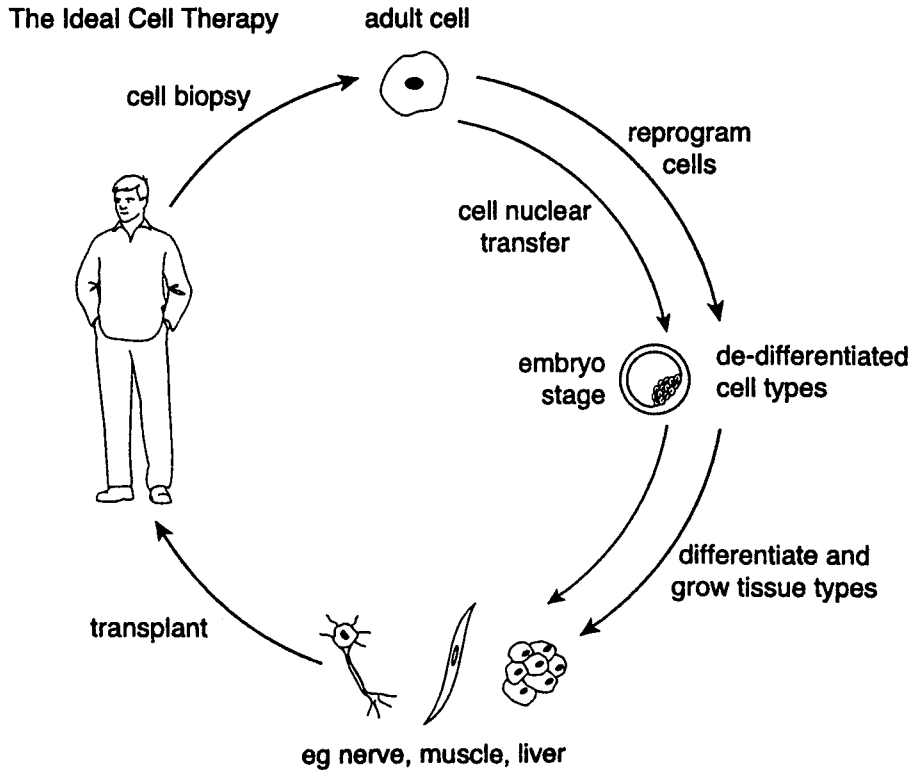


Figure 9. Summary of procedures.

The three main lines of research are summarised in this diagram. On the left, embryonic stem cells are recovered from the inner cell mass of surplus embryos from IVF programs, for research including cell therapies. In the centre, embryos are cloned by the somatic cell nuclear transfer technique to provide 'designer' stem cells for research aimed at specific patients and diseases. On the right, adult stem cells may be isolated, programmed to grow into particular cell or tissue types, and used in cell therapies.